

Figure 1

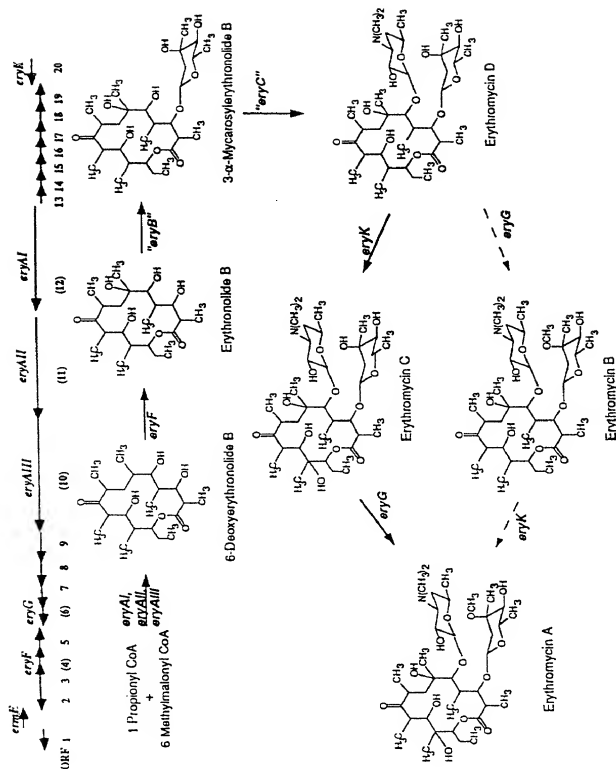


Figure 2

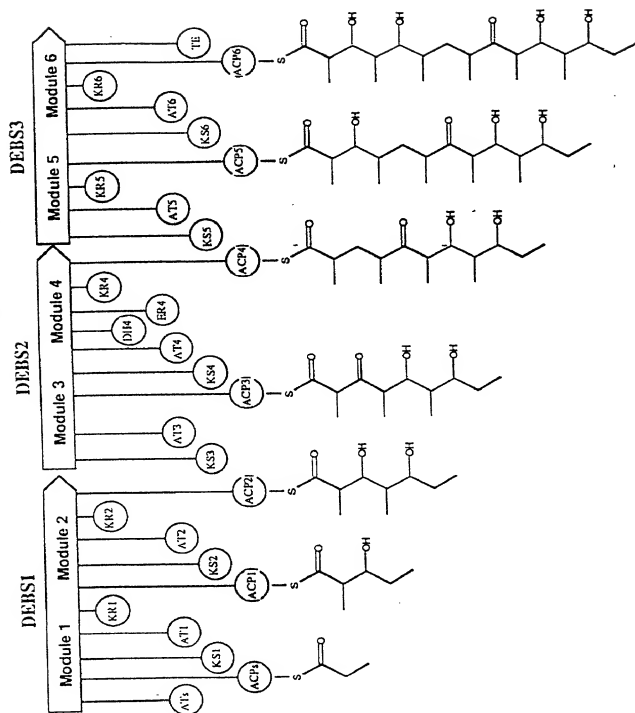


Figure 3

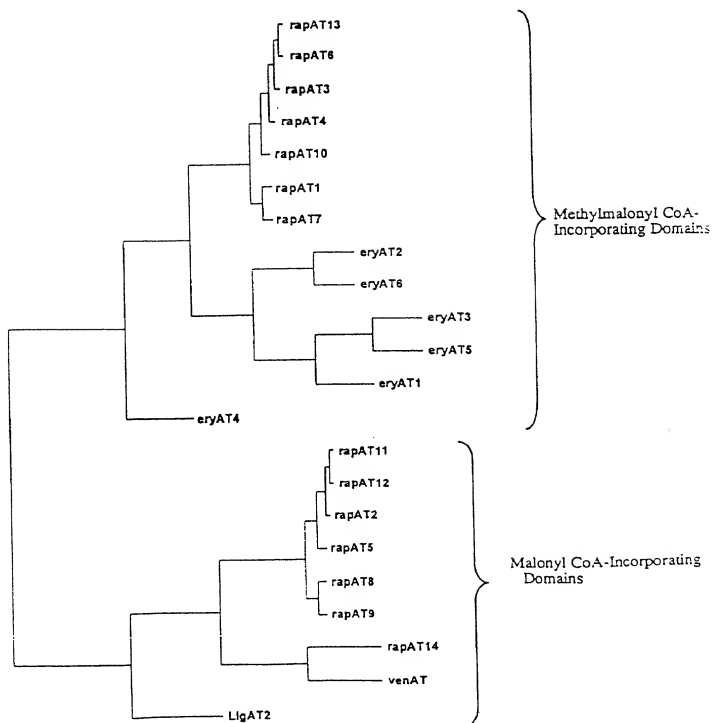
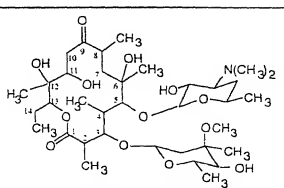
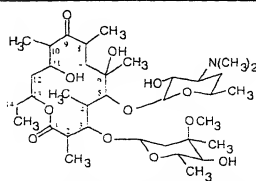
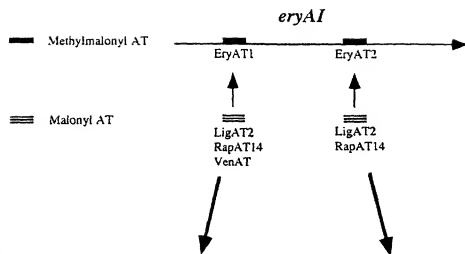
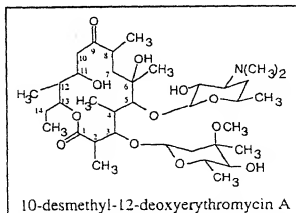


Figure 4a



and



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Figure 4b

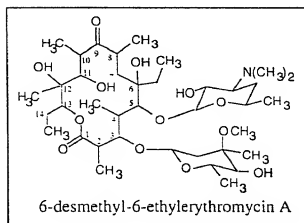
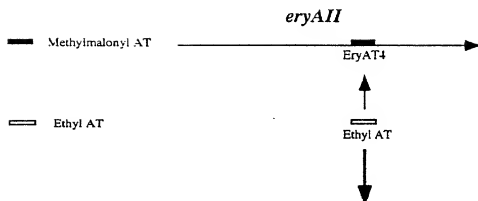


Figure 5

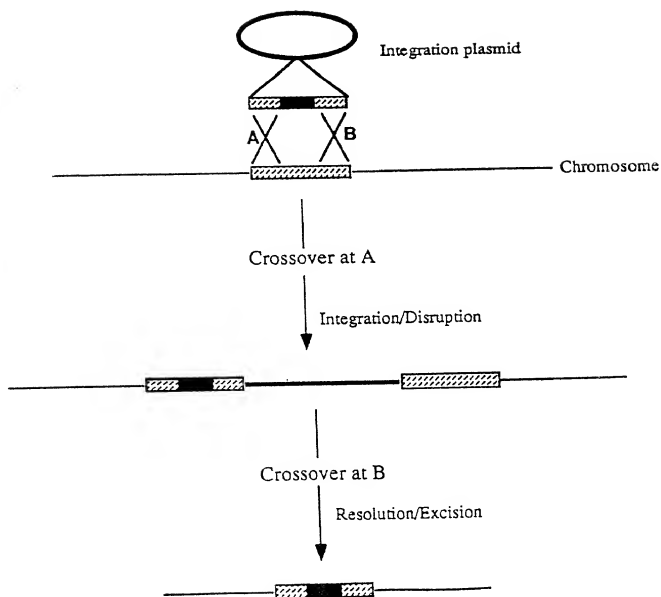


Figure 6

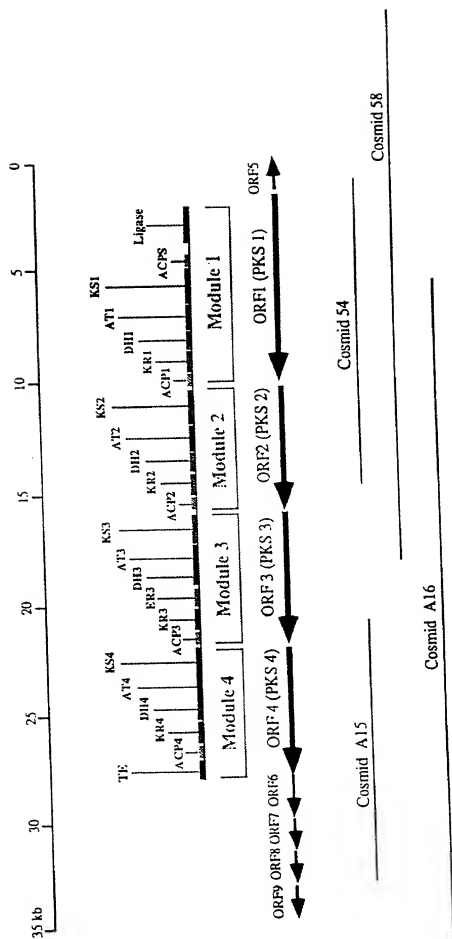


Figure 7

GGGGCGCTGGCGGTGATGTTACCGGACAGGGCTCCCAACGCCCGGCATGGGACGACAG 60
G P L A V M F T G Q G S Q R P G M G R Q 20
TTGTACGAGCACTTCCCCGTCTTCGCCCAGGCACTGGACGAGGTCTTCGCACTCGCCACC 120
L Y E H F P V F A Q A L D E V F A L A T 40
CCCGGACTACGC3AGGTGATGTTTCGACCCCGACAGGCGGAAACACTCCAACGCACCGAC 180
P G L R E V M F D P D Q A E T L Q R T D 60
CACGCCAGATCGCCCTGTTTCGCCTTCGAAACCGCCCTCTACCGACTCTGGGAATCCTGG 240
H A Q I A L F A F E T A L Y R L W E S W 80
GGCCTGCGACCCGACATGGTCTGCGGACACTCGGTTCGGAGAAATCACCGCAGCCACGTC 300
G L R P D M V C G H S V G E I T A A H V 100
TCCGGCACCCCTCACCTCCCCGACGCCGTCACCTCGTCACCACACGCGGCACCCCTCATG 360
S G T L T L P D A V H L V T T R G T L M 120
CAAAACCTGCCCCCGGGCGGCCATGCTCGCGCTCGCCACCGACCCCCACACCTCCAA 420
Q N L P P G G A M L A V A T D P H T L Q 140
CCCCACCTCGACAACCAACGACGACCATCTCCATCGCGCCCATCAACGSCCCCCACGCC 480
P H L D N H H D T I S I A A I N G P H A 160
ACCGTCTCTCGGGGACCGCACCCCTCCACCACATCGCCACCCAACTCAACACCAAA 540
T V L S G D R T T L H H I A T Q L N T K 180
ACCAACTGGCTCAAGCTAGCCAGCCCTTCCACTCCCCCTCATGCAACCCATCCTCCAA 600
T N W L N V S H A F H S P L M Q P I L Q 200
CCCTTCACCACCCCTCAACACCCCTCACCCACCAACCCCCACACACCCCTCATCAGC 660
P F T T T L N T L T H H P P H T P L I S 220
ATGCTACCGCCACACCCACCCCGACACCCCACTGGACCCAGCAGCATCACCGCA 720
M L T A T P T H P D T T H W T Q H I T A 240
CCCGTCGGGTACACCGACACCCCTCCACCACCTCCACCACCAACCGCATCACCACTACCTC 780
P V R Y T D T L H H L H H H G I T T Y L 260
GAAATCGGCCCCGACACCCCTCACCGCCCTCGCCCGACCAACCCCTCCCCACCAACCC 840
E I G P D T T L T A L A R T T L P T T T 280
CACCTCATCCCCACCAACCCCGCGCAACCAACGAAGTCCGAGCAGCAAGAGGGGTTG 900
H L I P T T R R N H N E V R S T N E A L 300
GGCAGGGTGTTCAGCTGGGCCACTCGGTGGACTGGCGGGCCCTCACTCCGACCGGGAGG 960
G R V F S V G H S V D W R A L T P T G R 320
CGTACCTCCCTGCGGACGTACCCCT 935
R T S L P T Y P 328

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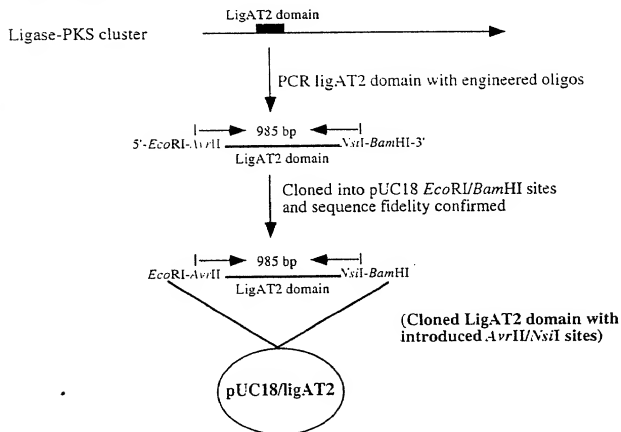
Figure 8

PCR oligos:

N-terminal Oligo: 5' *Eco*RI Tag-CCTAGG^{AvrII}GCTGGCGGTGATGTTCA-3'
GGGCC
 [Engineered *Avr*II] [Homologous region]

C-terminal Oligo: 5' *Bam*HI Tag-ATGCATACGTCGGCAGGGAGGTAC-3'
G GG^{NsiI}
 [Engineered *Nsi*I] [Homologous region]

PCR cloning:



09725056-12100

Figure 9

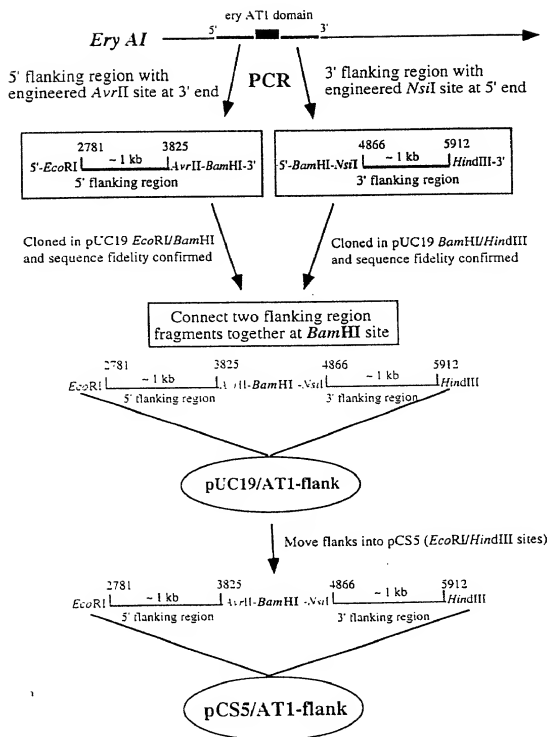


Figure 10

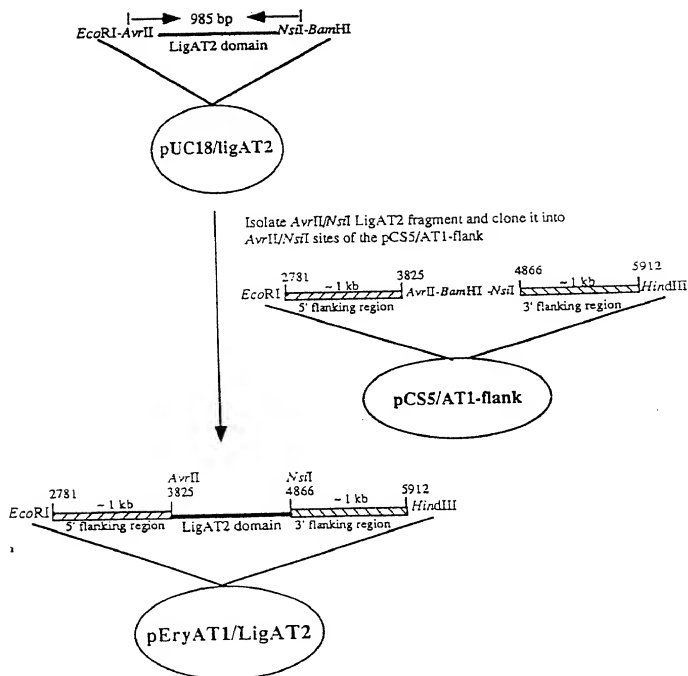


Figure 11

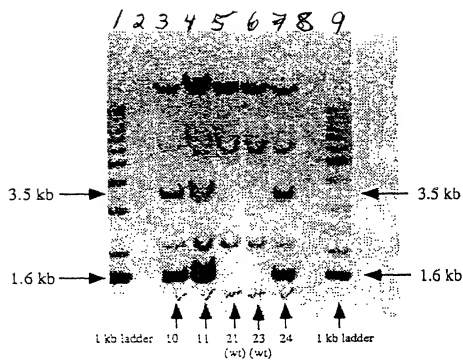
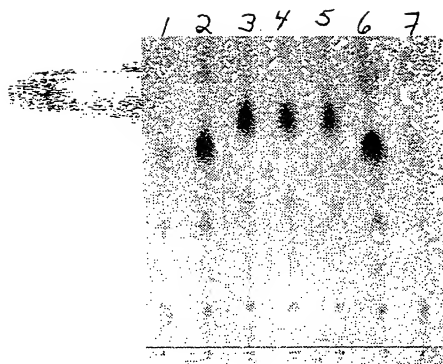


Figure 12



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Figure 13

Construction of eryAT2 flanking regions in pCS5

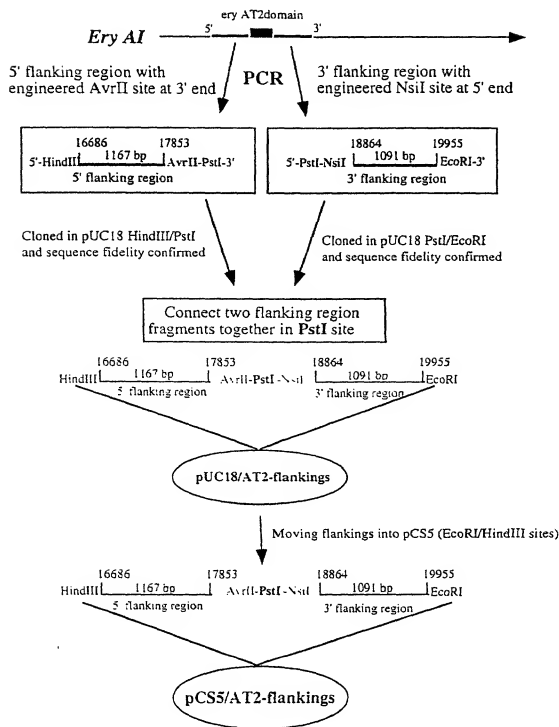


Figure 14

Scheme for Construction of pEryAT2/LigAT2 Integration Plasmid

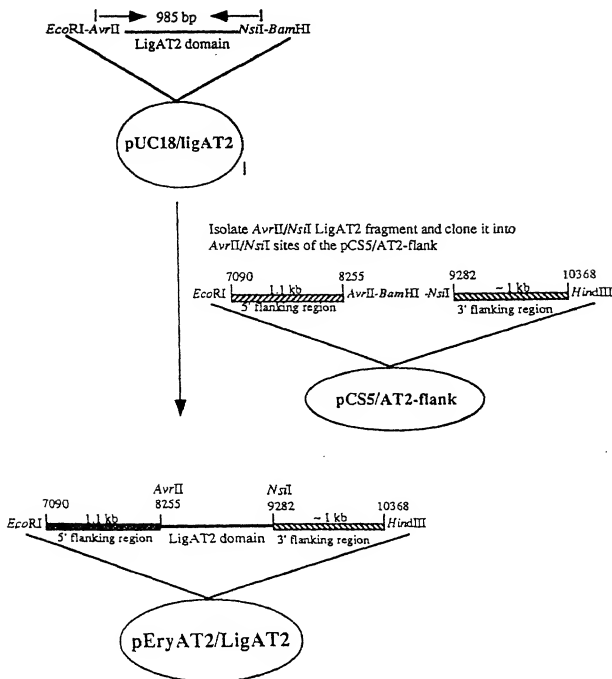


Figure 15

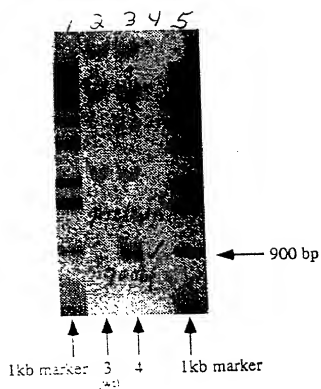
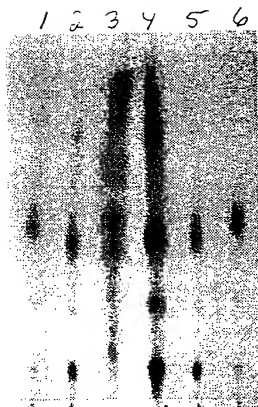


Figure 16



00735255.121100

Figure 17

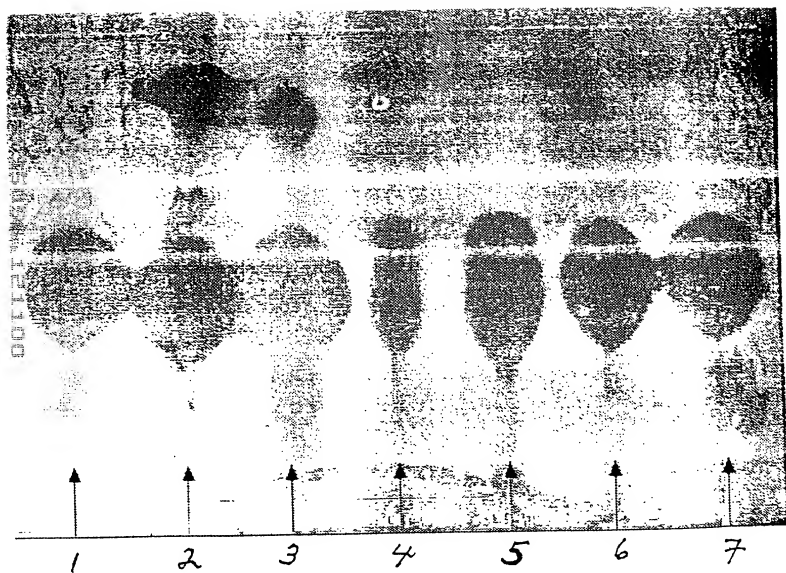


Figure 18

CCTAGGACGGCAGTCCCTGCTCACCAGGGCAGGGTTCCCAGCGTCAGGGCATGGGGCCGCGAA 60
 P R T A V L L T G Q G S Q R Q G M G R E 20
 CTGTACGACCGGTACCCGGTGTTCGGCCGCTCGTTTCGACGCGATCTGCGCTCAACTCGAC 120
 L Y D R S P V F A A S F D A I C A Q L D 40
 GGGCAACTGCCTCGTCCCTCAAGGACGTTCTCTTCGCCCCGAGGGGTCGGAGGACGCC 180
 G Q L P R P L K D V L F A P E G S E D A 60
 GCGCTCATCGACCGTACGGTGTTCACACAGGCGGCTCTGTTCGCCGTGGAGACCTCCCTG 240
 A L I D R T V F T Q A A L F A V E T S L 80
 TTCCGGCTGTTCGAGGCCACCGGCTCGTCCCCGACTACCTCATCGGCCACTCCATCGGC 300
 F R L F E A H G L V P D Y L I G H S I G 100
 GAAGTGACCGCGGCCACCTGGCCGGGGTCTCGATCTGGCGGACGCGTCGCGTCTGGTC 360
 E V T A A H L A G V L D L A D A C V L V 120
 GCCCACCGCGGCCCTGATGCAGTCGGCCCGGGCCGGCGCGCATGGCCGCGTCCAG 420
 A H R G R L M Q S A R A G G A M A A V Q 140
 GCGAGCGAGGACGAGGTACGCGAGGCCCTCGCGACCTTCGACGATGCGGTTGCCGTGGCC 480
 A S E D E V R E A L A T F D D A V A V A 160
 GGAGTCAACGCGCCGAACGCCACCGTCTGTCGCGCAGGAGGACGCGGTTCGAGCGGCTG 540
 G V N G P N A T V V S G D E D A V E R L 180
 GTCGCGCGCTGGCGGAGCAGGGCAGGCGGACGAAGCGGCTGCCGCTCAGCCACGCCTTC 600
 V A R W R E Q G R R T K R L P V S H A F 200
 CACTCGCCGCACATGGACGGGATCGTCGACGAGTTTCGTACCCGCGTCTCCGGGCTCACC 660
 H S P H M D G I V D E F V T A V S G L T 220
 TTCCGCTCCCGACGATCCCGTCTGTCACACGTCACCGGACCCCTGCCACCGTCGAC 720
 F R S P T I P V V S N V T G T L A T V D 240
 CAGCTGACCTCGCCCGGCTACTGGGCACGCCACATCCGCGAGGCCGTGCGCTTCGCCGAC 780
 Q L T S P A Y W A R H I R E A V R F A D 260
 GGGGTGCGGTACCTGGAGGGCGAGGGCGTCACCGAATGGCTGGAGCTCGGGCCCGACGGC 840
 G V R Y L E G E G V T E W L E L G P D G 280
 GTTCTCGTCGCCCTGGTCGAGGACTGCCTGGCGAAGGAGGCGGGATCGCTCGCGTCCGCC 900
 V L V A L V E D C L A K E A G S L A S A 300
 CTGCGCAAGGGGGCAGGCGAGCCCCACACCGTGGGCGCGGCCATGGCCCCGCGCGGTGCTG 960
 L R K G A S E P H T V G A A M A R A V L 320
 CGCGGATCCGGCCCCGACTGGGCGGCGGTGTTCGCCGGCGCACGGCGGGTCGACCTTCGG 1020
 R G S G P D W A A V F P G A R R V D L P 340
 ACGTATGCAT 1030
 T Y A 343

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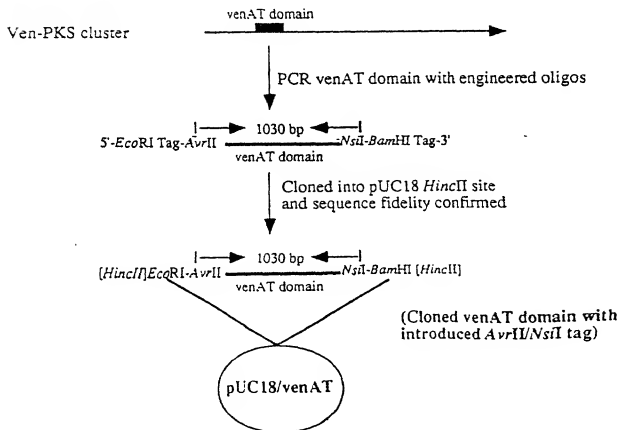
Figure 19

PCR oligos:

N-terminal Oligo: 5' *EcoRI* Tag-^{AvrII}CCTAGGACGGCAGTCCTGCTCACC-3'
GGCC
[Engineered *AvrII*] | [Homologous region]

C-terminal Oligo: 5' *BamHI* Tag-^{NsiI}ATGCATACGTCGGAAGTCGACCCG-3'
C C
[Engineered *NsiI*] | [Homologous region]

PCR cloning:



0073005-121100

Figure 20

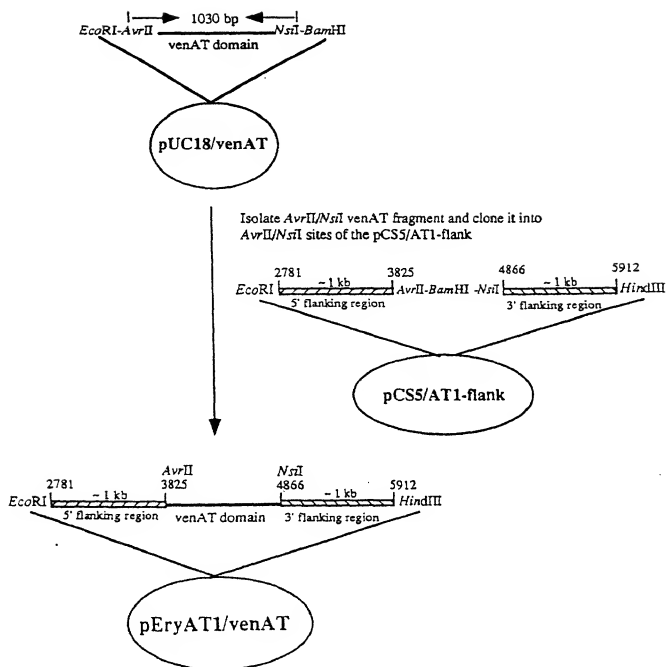


Figure 21

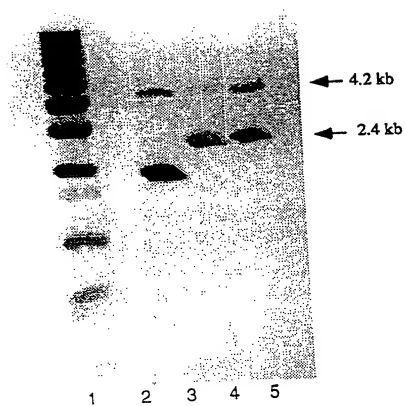
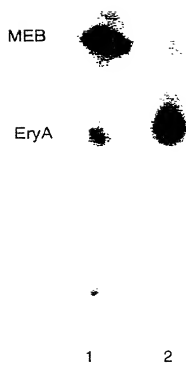


Figure 22



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Figure 23

PCR oligos:

N-terminal Oligo: 5' *EcoRI* Tag-CCTAGGTTGCCTTCCTGTTTCGAC-3'
 GGC C
 Engineered *AvrII* Homologous region

C-terminal Oligo: 5' *HindIII* Tag-ATGCATAGACCGGCAGATCCACCG-3'
 C G
 Engineered *NsiI* Homologous region

PCR cloning:

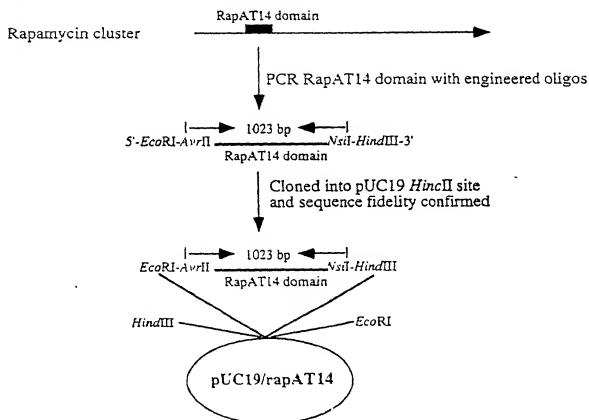


Figure 24

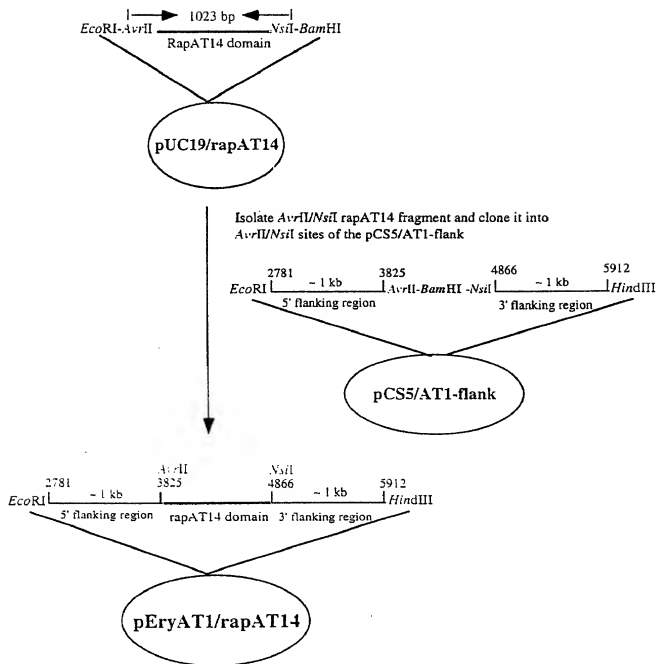
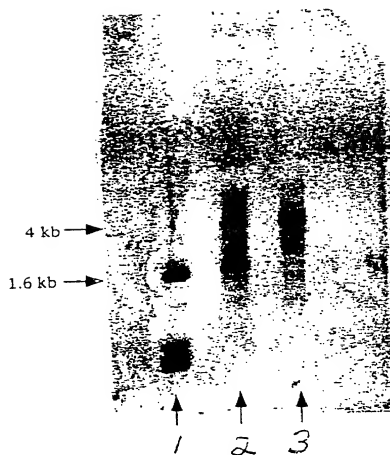


Figure 25



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Figure 26



09735056.121100

Figure 27

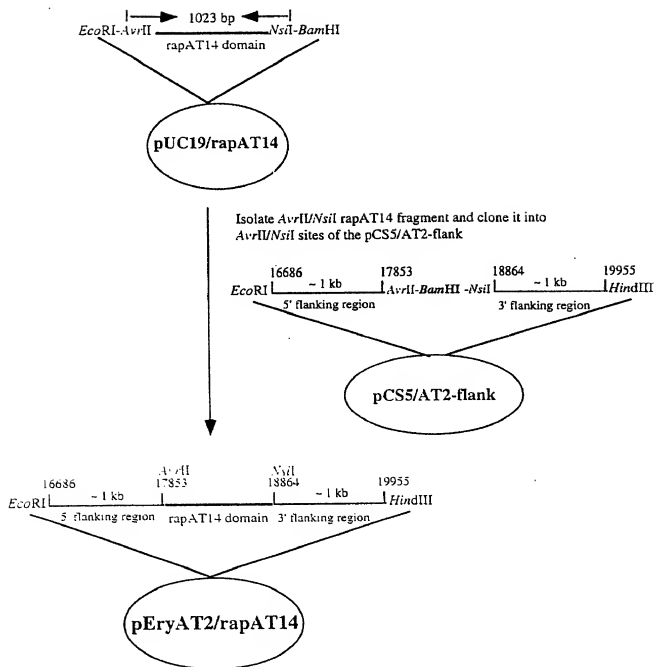
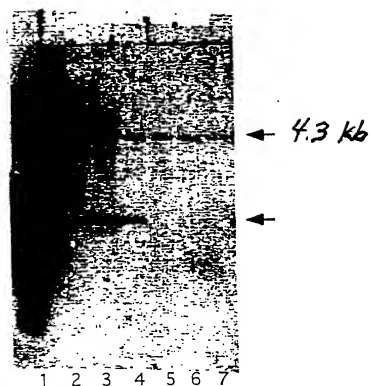
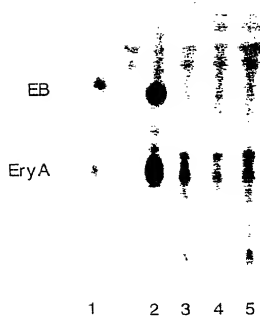


Figure 28



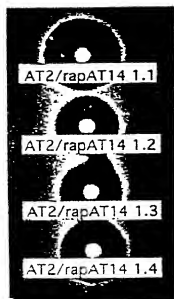
09735056.121100

Figure 29



09735056.121400

Figure 30



09735056-12340

Figure 31

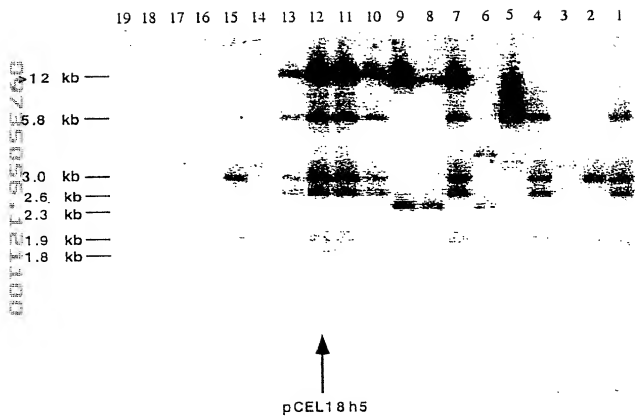


Figure 32

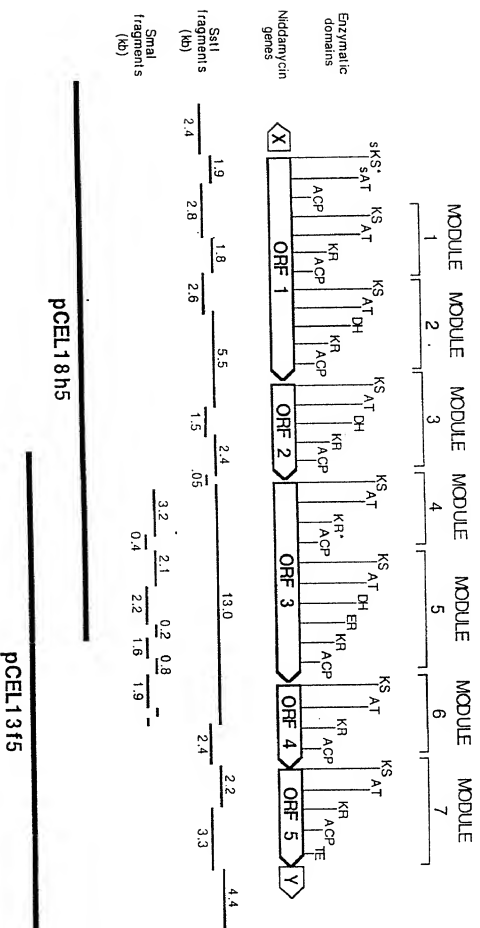


Figure 33

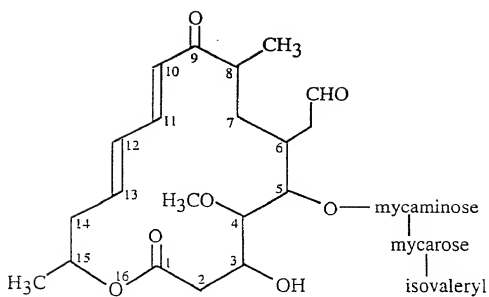


Figure 34

GCGCACCCTGTCGTGTTCTGTTCCCGGCCAGGGCTCGCAGTGGGCCGGAATGGCCGAG 60
 A D R ' / V F V F P G Q G S Q W A G M A E 20

 GGGCTGCTGGAGCGGTCCGGCGCTTCGGGAGTGGGCCGACTCGTGCAGCGCCGCGCTG 120
 G L L E R S G A F R S A A D S C D A A L 40

 CGGCCGTACCTCGGCTGGTCCGTGCTGAGCGTCTGCGCGGGGAACCGGACGCGCCCTCG 180
 R P Y L G W S V L S V L R G E P D A P S 60

 CTCGACCGGGTCGACGCTCGTGCAGCCGGTCTGTTTACGATGATGCTCTCGCTCGCGGCG 240
 L D R V D V V Q P V L F T M M V S L A A 80

 GTCTGGCGTGCCTGGGGGTGGAACCGCGCGCGGTCTGTCGGGCACTCGCAGGGTGAATC 300
 V W R A L G V E P A A V V G H S Q G E I 100

 GCGCTGCCCCATGTCGGCGGTGCGCTGCTGCTGGACGACTCGGCCCGGATCTGCGCCCTG 360
 A A A H V A G A L S L D D S A R I V A L 120

 CGCAGTCTGGCGGTGGCTCGGACTGGCGGGCAAGGGCGGCATGCTGGCGGTGCGCATGCGG 420
 R S R A W L G L A G K G G M V A V P M P 140

 CGCGAGGAGCTCGCGCCCGGGTGGTGAAGTGGGGGACCGTCTGCGCGCTCGCGCCGCTC 480
 A E E L R P R L V T W G D R L A V A A V 160

 AACAGCCCGGTCTCTGCGCGCTGCGAGGCGACCCGGAAGCGGCTGGCCGAACCTGGTGGCG 540
 N S P G S C A V A G D P E A L A E L V A 180

 CTGCTGACCGGTGAGGGGGTGCAGCCCGGGCGATCCCGCGCTGCAGCAGGCGGGCCAC 600
 L L T G E G V H A R P I P G V D T A G H 200

 TCGCCGACGGTGGAGCGCTGCGGGGTCTCTGCTGGAGGTGCTGGCCCGGTGCGCCCC 660
 S P Q V D A L R A H L L E V L A P V A P 220

 CGACCGCGCGACATCCCGTTCTACTCGACGCTGACCGCGGGGTGCTGGACCGCACCGAG 720
 R P A D I P F Y S T V T G G L L D G T E 240

 CTGGACGCGACGTACTGGTACCGCAACATGCGCGAGCCCGTCTGAGTTCGAGCGGGCCACA 780
 C D A T Y W Y R N M R E P V E F E R A T 260

 CGGGCGCTGATCGCGGACGGGCACGACGTCTTCTGGAGACGAGCCCGCATCCCATGCTG 840
 R A L I A D G H D V F L E T S P H P M L 280

 GCGCTGGCGCTGGAGCAGAGGTACCGGACCGCGGCAACGACGCGCGGTGCTCGGGACC 900
 A V A L E Q T V T D A G T D A A V L G T 300

 CTGCGCCCGCGGACCGCGGTCTCTCGCGGCTGGCCCTGGCGGTGCGCGCGCTTCGCGG 960
 L R R G H G G P R A L A L A V C R A F A 320

 CACGCGCTGGAGGTGAGACCCCGAGGCGGTCTTCTGGTCCGGGCGCACCGCCCGCTGGAGTTG 1020
 H G V E V D P E A V F G P G A R P V E L 340

 CCCACCTATCG 1032
 P T Y P 344

09735056-121100

Figure 35

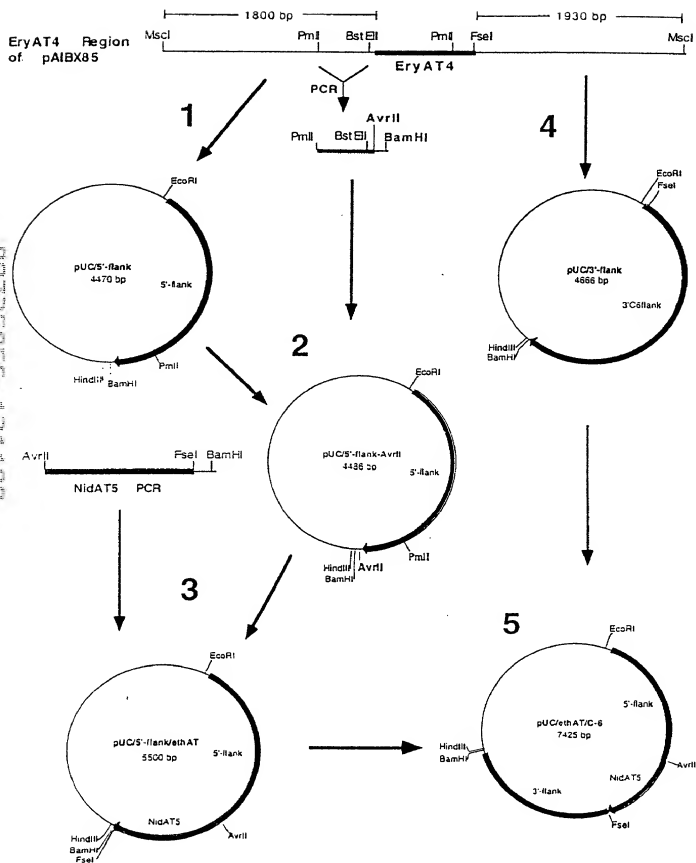


Figure 36

Protein Sequence	S A P R K P
Original Sequence	TCCGCGCCGCGCAAGCCG
Altered Sequence	TCCGCGCC TAGGA AGCCG

AvrII site

PCR Oligos for 5'-flank AvrII site

N-Terminal oligo (Seq 10 no 21) 5'-GAGAGAGGAACCAACGCGCACGTGATCGTCGAAGAGGCACCAGC
 (5' flank sequence) (PmlI site)

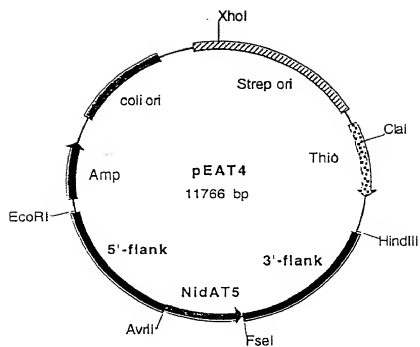
C-terminal oligo (Seq 10 no 22) 5'-GAGAGAGGATCCGACCTAGGCGCGGAGGTCACCGGCGCGACGGCG
 (BamHI site) (AvrII site) (5' flank sequence)

PCR oligos for NidAT5 fragment

N-Terminal oligo (Seq 10 no 23) 5'-GAGAGACCTAGGAAGCCGGTGTTCGTGTTCCCCGGCCAGGGCT
 (AvrII site) (Beginning of NidAT5)

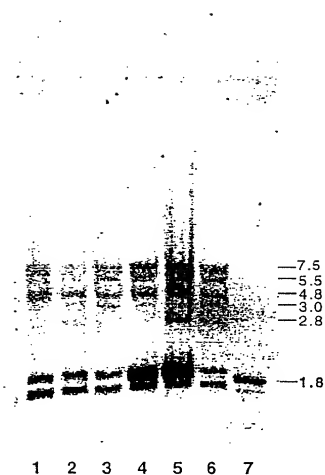
C-terminal oligo (Seq 10 no 24) 5'-GAGAGAGGATCCGAGGCCGGCCGTGCGCCCCGACCGAAGACCGCCTC
 (BamHI site) (FseI site) (3' end of NidAT5)

Figure 37



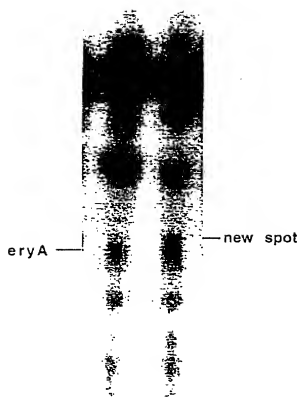
09735056-121100

Figure 38



00735056.121100

Figure 39



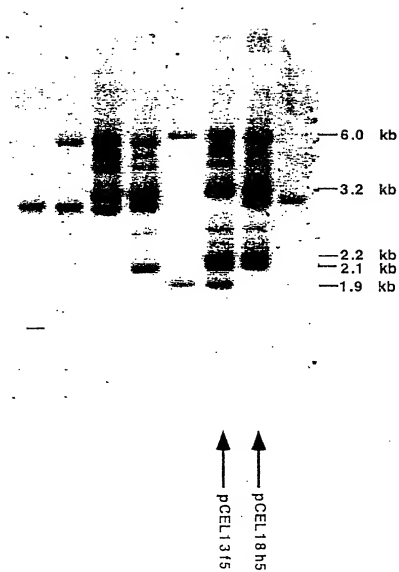
A) SCM only

B) SCM + 50mM butyric acid

A

B

Figure 40



09735056.121100

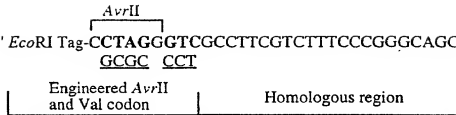
Figure 41

CGCGCGCCTGCCTTCGTCTTCCCGGGCAGGGCGCCAGTGGGCGGACTGGGAGCGCGG 60
 R A P A F V F P G Q G A Q W A G L G A R 20
 CTCCTCGCGGACTCCCCCGTCTTCCGCGCCAGGGCCGAGGCATGCGCGCGGGCGCTGGAG 120
 L L A D S P V F R A R A E A C A R A L E 40
 CCTCACCTCGACTGGTCGGTCTCGACGTGCTGGCGGGCGCCCCGGGACCCCTCCCATC 180
 P H L D W S V L D V L A G A P G T P P I 60
 GACCGGGCCGACGTGGTGCAGCCGGTGCTGTTACCCACGATGGTCTCGCTGGCCGCCCTC 240
 D R A D V V Q P V L F T T M V S L A A L 80
 TGGGAGGCCACGGGGTCGGCCGGCCGGTCGTGGGCGACTCCACGGGCGAGGTGGCC 300
 W E A H G V R P A A V V G H S Q G E V A 100
 GCGGCCCTGCGTGGCCGGTGCCCTGCTGCTGGACGACGTGCCCTGGTGATCGCCGGACGC 360
 A A C V A G A L S L D D A A L V I A G R 120
 AGCAGGCTGTGGGGGCGGTGGCCGGGAACGGCGGGATGCTCGCGGTGATGGCTCCGGCC 420
 S R L W G R L A G N G G M L A V M A P A 140
 GAGCGGATCCGTGAGCTGCTCGAACCATGGCGGCAGCGGATTTCGGTGGCGGCGGTCAAT 480
 E R I R E L L E P W R Q R I S V A A V N 160
 GGCCCCGCCCTCGGTACCGTCTCCGGTGACGCGCTCGCGTGGAGGAGTTCGGCGCGCGG 540
 G P A S V T V S G D A L A L E E F G A R 180
 CTCTCCGCGAGGGGGTGCTGCGCTGGCCGCTGCCGGCGTTCGACTTCGCGGCCACTCG 600
 L S A E G V L R W P L P G V D F A G H S 200
 CCGCAGGTGGAGGAGTTCGCGGCTGAGCTCCTGGACCTGCTCTCCGGCGTACGCCCGGT 660
 P Q V E E F R A E L L D L L S G V R P A 220
 CCTTCGCGGATACCTTCTTCTCCACCGTGACGGCGGGTCTTTCGCGCGCGGACACGCTG 720
 P S R I P F F S T V T A G P C G G D Q L 240
 DCGGGGCGGTACTGGTACCGCAACACGCGGAACCCGTGGAGTTCGACGCCACGGTCCGG 780
 A A I D E I A A D E G V A A T A L H T L 260
 GCGCTGCTGCGTGCGGGCCATCACAGTTTCATCGAGGTGCGTCCGCATCCGTGCTCAAC 840
 A L L R A G H H T F I E V G P H P L L N 280
 GCCGCGATCGACGAGATCGCAGCGGACGAGGGGTAGCGGCCACGGCCCTGCATACGCTC 900
 A A I D E I A A D E G V A A T A L H T L 300
 CAGCGGGGCGCTGGCGGCCCTTGACCGCGTGCAGCAACCGGTTGGCGCCGCTTTCGCGCAC 960
 Q R G A G G L D R V R N A V G A A F A H 320
 GGTGTCCGGGTGCACTGGAACGCCCTGTCTCAGGGCACCGGTGCGCGCAGGGTGC CGCTT 1020
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 CCCTCGTACGCTTC 1035
 P S Y A F 345

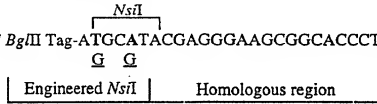
09735056.121100

U.S. DEPARTMENT OF JUSTICE

N-terminal Oligo: 5' *Eco*RI Tag-CCTAGGGTCGCCTTCGTCTTCCCGGGCAGG-3'
GCGC CCT



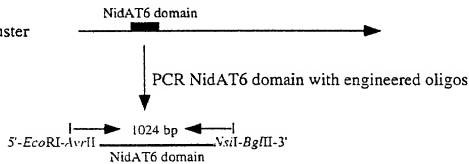
C-terminal Oligo: 5' *Bgl*III Tag-ATGCATACGAGGAAGCGGCACCTGC-3'



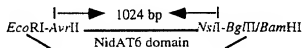
Niddamycin cluster

NidA6 domain

PCR NidA6 domain with engineered oligos



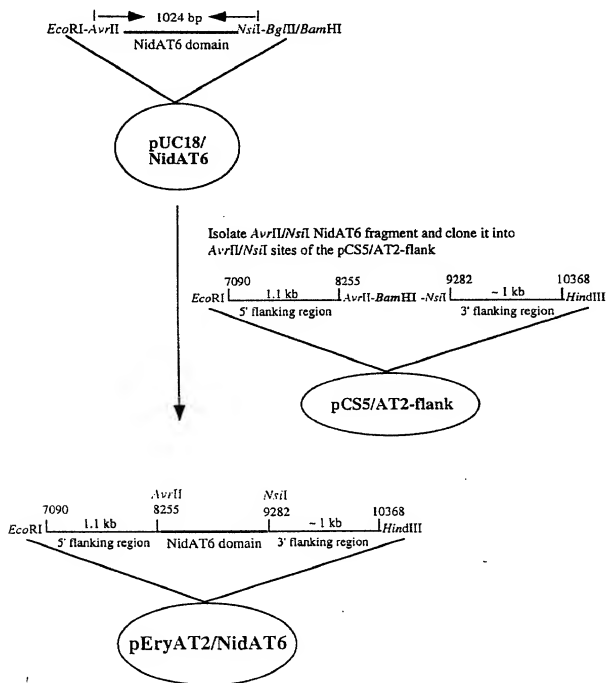
Cloned into pUC18 *Eco*RI-*Bam*HI sites
and sequence fidelity confirmed



(Cloned NidAT6 domain with introduced *AvrII*/*NsiI* sites)



Figure 43



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